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Full Length Research Paper

Chemical composition and antioxidant activity of *Euphorbia fischeriana* essential oil from China

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The chemical composition of *Euphorbia fischeriana* essential oil from north China was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS), 43 components were identified. *β*- Eudesmol (18.22%), *p*-Menth-8-en-2-ol (9.36%), Caryophyllene oxide (8.61%), *β*-Selinenol (6.83%), Cedrol (4.30%) and *β*-Selinene (4.21%) were the main components in the oil of *E. fischeriana*. The antioxidant activity of *E. fischeriana* essential oil determinated spectrophotometrically with 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), indicated an IC_{50} value of 57.24 µg/ml.

Key words: *Euphorbia fischeriana*, essential oil, antioxidant activity.

INTRODUCTION

Euphorbia fischeriana Steud (Euphorbiaceae) is a perennial herbaceous plant distributed mainly in north China, and belongs to the large family of Euphorbiaceae. Euphorbia genus is the most representative of the family with more than 1600 species (Ozenda, 1991). Plants of this genus are known for their rich content in secondary metabolites. Indeed, numerous studies undertaken on this genus have revealed presence of triterpenes (Lima et al., 2003), diterpenes (Shi et al., 2005), macrocyclic diterpenes (Rédei, 2003), steroids (Tanaka et al., 1999) and aromatic compounds (Öksüz et al., 2002). The root part of the plant is known as `Lang Du' in traditional Chinese medicine and classified as a “toxic drug” due to its high potency and relatively violent pharmacological effects. It is used with great care for the treatment of edema, indigestion, cough, asthma and chronic bronchitis (Jiangsu New Medical College, 1977).

The aim of the present study was to investigate the chemical composition and antioxidant of *E. fischeriana* essential oil. To the best of our knowledge, this is the first report on the chemical composition and antioxidant activity of *E. fischeriana* essential oil from north China.

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MATERIALS AND METHODS

Plant material

Dried roots of E. fischeriana were purchased from the Shanghai Chinese medicinal herbs corporation and identified by Professor Wang Zhen-Yu, Northeast Forestry University. The voucher specimen (No. EF101021) has been deposited in the Herbarium of School of Food Science and Engineering, Harbin Institute of Technology.

Essential oil extraction

The root parts of *E. fischeriana* were left to dry at room temperature and 500 g of the plant material were coarsely minced and placed in a flask containing 3000 ml of water and steam distilled in a Clevenger-type apparatus for 2.5 h. The essential oils (yield: 1.52% from *E. fischeriana*) were obtained using *n*-hexane as collecting solvent and were dried over anhydrous sodium sulphate and kept at -4 ℃ until it was analyzed by GC-FID and GC-MS.

Chemical analysis

The essential oil samples were analyzed by gas chromatography (GC) and by gas chromatography-mass spectrometry (GC/MS). The oils were analyzed by using Agilent 6890N gas chromatograph (DB-5 column, 30 m × 0.25 mm; 0.25 pm film thickness, FID). The oven temperature was held at 60℃ and then programmed from 60 to 270℃ at a rate 3℃/min, using Helium as the carrier gas (1.0 ml/

Table 1. Percentage component of the essential oils from *E. fischeriana*

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Compound	Contents (%)	RI	Identification
α-Pinene	1.36	944	RI_1, MS
Camphene	0.52	959	RI_1, MS
β-Pinene	tr.	978	RI_1, MS
β-Cymene	1.19	984	RI_1, MS
D-Limonene	tr.	1037	RI_1, MS
Eucalyptol	0.90	1046	RI_1, MS
Fenchol	1.29	1123	RI_1, MS
α-Campholenal	1.10	1134	RI_1, MS
L-Pinocarveol	0.83	1152	RI_1, MS
Borneol	2.57	1178	RI_2
4-Carvomenthenol	1.91	1192	RI_1, MS
p-Menth-8-en-2-ol	9.36	1202	RI_1, MS
Myrtenol	1.24	1207	RI_1, MS
D-Carvone	tr.	1242	RI_1, MS
trans-Myrtanol	1.29	1264	RI_1, MS
trans-Anethol	0.40	1267	RI_1, MS
2,4-Decadienal	1.13	1284	RI_1, MS
α-Copaene	1.36	1370	RI_2
β-Panasinsene	0.32	1378	RI_1, MS
α- Gurjunene	0.12	1393	RI_1, MS
α-Cedrene	2.96	1407	RI_1, MS
Thuiopsene	1.17	1410	RI_1, MS
β-Caryophyllene	2.88	1414	RI_1, MS
β-Gurjunene	1.15	1449	RI_1, MS
α-Curcumene	1.10	1480	RI_1, MS
β-Chamigrene	3.50	1488	RI_1, MS
β-Selinene	4.21	1492	RI_2
α-Selinene	3.14	1497	RI_1, MS
Cuparene	2.07	1501	RI_1, MS
α-Muurolene	1.15	1505	RI_1, MS
Calamenene	tr.	1538	RI_1, MS
Elemol	1.54	1557	RI_1, MS
Caryophyllene oxide	8.61	1573	RI_2
Isoaromadendrene oxide	tr.	1590	RI_1, MS
Alloaromadendrene oxide	tr.	1595	RI_1, MS
Cedrol	4.30	1596	RI_1, MS
β- Eudesmol	18.22	1654	RI_1, MS
β-Selinenol	**6.83**	1657	RI_1, MS
Rimuen	0.46	2011	RI_1, MS
Sclaren	0.13	2032	RI_1, MS
Kaurene	1.43	2045	RI_1, MS
Abietadiene	1.49	2088	RI_2
Abietatrien	0.12	2094	RI_2
Monoterpene hydrocarbons	3.07		
Oxygenated monoterpenes	22.02		
Sesquiterpene hydrocarbons	25.13		
Oxygenated Sesquiterpene	39.50		
Diterpene hydrocarbons	3.63		
Total identified	93.35		

tr: Traces (<0.05%); RI: retention index according to n-alkanes (C5–C25) on the DB-5 column; MS: mass spectra data; RI1: retention data according to literature values; RI2: retention data according to authentic standards.

Table 2. Radical scavenging activity of the oils of *E. fischeriana*, BHT and ascorbic acid with DPPH.

Material	IC_{50}[a]
The oil of *E. fischeriana*	57.24±2.31
BHT	26.05±1.87
Ascorbic acid	63.15±3.28

[a]Concentration (µg/ml) for a 50% inhibition. Values represent average of triplicates ± standard deviation.

min). Injector and detector temperatures were 250°C. Quantitative data were obtained by electronic integration of peak areas without the use of correction factors.

Analyses by GC/MS were performed using a chromatograph agilent 6890 interfaced to an agilent 5973 mass spectrometer system operating in the EI mode at 70 ev, equipped with a split/splitless injector. The transfer line temperature was 250°C. Helium was used as the carrier gas (1 ml/min) and the capillary column used was DB-5 MS column (30 m × 0.25 mm; 0.25 µm film coating). The temperature programme was the same as that used for the GC-FID analysis; split ratio 1:10; scan time, 1 s; mass range, 50 to 500 amu. The injected volume was 1 µl.

Qualitative and quantitative analyes

Most constituents were identified by gas chromatography by comparison of their GC retention indices (RI) with those reported in literature (Adams, 1995) or with those of standards purchased, synthesized or identified in oils of known composition. Further identification was confirmed when possible (percentage of similarity > 80%) by comparison of their mass spectra with those stored in MS databases (NIST and Wiley libraries). Relative component concentrations were obtained directly from GC peak areas and appear in Table 1 as percentage composition.

Antioxidant activity

The antioxidant activity of *E. fischeriana* essential oil was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams et al., 1995). A methanolic stock solution (50 ml) of the antioxidant was placed in a cuvette and 2 ml of 6×10^{-5} M methanolic solution of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined by Pgeneral-T6 spectrophotometer after 1 h for all samples.

Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant, that is, the control, was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution (Blois, 1958).

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated (Yen and Duh, 1994).

RESULTS AND DISCUSSION

Table 1 lists the identified constituents, representing the chemical composition of the oil. The constituents were identified by comparison of their mass spectra with those in the computer library or with the indice of retention reported in literature.

The volatile constituents identified in the oils of *E. fischeriana* are listed in Table 1 in the order of their elution from the DB-5 MS column. Essential oil yield of *E. fischeriana* was found to contain 43 components representing 93.35% of the oil. The main constituents of *E. fischeriana* oil were *β*-Eudesmol (18.22%), *p*-Menth-8-en-2-ol (9.36%), Caryophyllene oxide (8.61%), *β*-Selinenol (6.83%), Cedrol (4.30%) and *β*-Selinene (4.21%). Essential oil of *E. fischeriana* was characterized by the preponderance of sesquiterpene (64.63%), whereas the monoterpene and diterpene were presented in lower amounts (25.09 and 3.63%, respectively).

Relatively stable organic radical DPPH· has been widely used in the determination of the antioxidant activity of single compounds as well as the different plant extracts. The method is based on the reduction of alcoholic DPPH· solutions in the presence of a hydrogen donating antioxidant. DPPH· solutions show a strong absorption band at 517 nm appearing a deep violet color. The absorption vanishes and the resulting de-colourization is stoichiometric with respect to degree of reduction. The remaining DPPH·, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. The method was used to evaluate the antioxidant properties of *E. fischeriana* essential oil in comparison with those of known natural and synthetic antioxidants, ascorbic acid and BHT (Table 2).

As can be seen from the table, IC_{50} value of the *E. fischeriana* essential oil was determined as 57.24 ± 2.31 µg/ml. When compared to BHT and ascorbic acid, oil has been found less effective than BHT, but more effective than ascorbic acid.

In this study, the essential oil of *E. fischeriana*, was found to possess remarkable radical-scavenging. The bioactive components of *E. fischeriana* oil can act as primary and secondary antioxidants, scavenging free radicals, and can therefore inhibit the lipid peroxidation. Selected active constituents of *E. fischeriana* essential oil may be an alternative to more toxic synthetic antioxidants as additives in food, pharmaceutical and cosmetic preparations.

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